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McBain, Andrew J; Chapple, Iain

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Consumer Safety Considerations of Skin and Oral Microbiome Perturbation

Andrew J McBain[a], Catherine A O'Neill[a], Alejandro Amezquita[b], Laura J Price[b], Karoline Faust[c], Adrian Tett[d], Nicola Segata[d], Jonathan R Swann[e], Adrian M Smith[f], Barry Murphy[g], Mike Hoptroff[g], Gordon James[f], Yugandhar Reddy[h], Anindya Dasgupta[h], Tom Ross[i], Iain L Chapple[j], William G. Wade[k] and Judith Fernandez-Piquer[b*]

[a]The University of Manchester, UK

[b]Unilever, Safety & Environmental Assurance Centre (SEAC), Colworth Science Park, Sharnbrook, UK

[c]KU Leuven Department of Microbiology and Immunology, Rega Institute, Laboratory of Molecular Bacteriology, Leuven, Belgium

[d]Centre for Integrative Biology, University of Trento, Trento Italy

[e]Division of Integrative Systems Medicine and Digestive Diseases, Imperial College London, UK

[f]Unilever, Colworth Science Park, UK

[g]Unilever, Port Sunlight, UK

[h]Unilever, Bangalore, India

[i]University of Tasmania, Tasmania, Australia

[j]The University of Birmingham, UK

[k]Centre for Host-Microbiome Interactions, Kings College London, UK

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*Current address: Upfield, Rotterdam, The Netherlands

For correspondence: Andrew J McBain, Division of Pharmacy and Optometry, Faculty of Biology, Medicine and Health, The University of Manchester, Oxford Road, Manchester M13 9PT, UK. Tel: 44 161 275 2360; Email: andrew.mcbain@manchester.ac.uk

Summary

Microbiomes associated with human skin and the oral cavity are uniquely exposed to personal care regimes. Changes in the composition and activities of the microbial communities in these environments can be utilised to promote consumer health benefits; for example by reducing the numbers, composition or activities of microbes implicated in conditions such as acne, axillary odour, dandruff and oral diseases. It is however important to ensure that innovative approaches for microbiome manipulation do not unsafely disrupt the microbiome or compromise health, and where major changes in the composition or activities of the microbiome may occur, these require evaluation to ensure that critical biological functions are unaffected. This article is based on a two-day workshop held at SEAC Unilever, Bedford, United Kingdom, involving 31 specialists in microbial risk assessment, skin and oral microbiome research, microbial ecology, bioinformatics, mathematical modelling and immunology. The first day focused on understanding the potential implications of skin and oral microbiome perturbation, while approaches to characterise those perturbations were discussed during the second day. This article discusses the factors that the panel recommend are considered for personal care products that target the microbiomes of the skin and the oral cavity.

INTRODUCTION

The human microbiome

The last two decades have seen the effective application of culture-independent methods to study the human microbiota (the microbial cells) or microbiome (the associated DNA) (1). This has led to a deeper and more comprehensive analysis of the diverse range of organisms that inhabit the body, where a substantial proportion are not readily amenable to culture (2). In the process some but certainly not all knowledge gaps have been addressed. High-throughput sequencing is currently performed using a range of platforms including Illumina and Ion Torrent, which can rapidly sequence millions of fragments of DNA in parallel (3). Hypervariable regions of the bacterial 16S rRNA genes, or whole genome DNA is targeted to analyse complex microbial communities. For 16S amplicon sequencing in particular, bioinformatic analyses have been applied to cluster the generated sequences according to their similarity to define different operational taxonomic units (OTU), which are then compared to databases to reveal community composition. However, tools such as DADA2 are being increasingly used to obtain exact sequence variants (4) giving greater resolution (5). The often short sequencing reads and the large data volumes generated through NGS presents challenges and taxonomic classification and relative abundances can vary depending on the

bioinformatic pipeline used (3). Microbiome research has nevertheless identified considerably greater microbial diversity than had been previously characterised, overcoming some of the limitations of culture including issues of non-culturability. Whilst microbiome research in humans has focussed primarily on the gut, studies of the oral cavity (6-9) and skin (10-14) have facilitated the deeper understanding of these sites, which are of particular relevance to personal care. The use of personal care products can result in changes in microbiome that may be intentional or otherwise. It is however important to note that "oral microbiome" and "skin microbiome" are simplified terms referring to biogeography-dependent sets of communities where microbial composition and activities can vary markedly depending on site.

The challenge of establishing causality

The human microbiome provides protection against pathogenic organisms (14) and can stimulate the immune system (15, 16) and participate in the maintenance of different ecological niches present in the body (17). Fluctuations in micobiome composition may therefore perturb beneficial microbial functions with potential health implications for the host. The following section will consider some notable diseases of the skin and the oral cavity where differentiating between cause and association for microbiome composition has been challenging.

Atopic dermatitis (AD) is a chronic, relapsing inflammatory condition characterised by pruritis (itchiness), wheels and flares, and in severe cases, broken, bleeding skin. A high *Staphylococcus aureus* load has been reported to correlate with AD flares and *vice versa* in clinical studies involving AD patients, where coagulase negative staphylococci (CoNS) were more abundant in healthy controls (18). Colonisation with commensal staphylococci early in life appears to be protective against the development of AD (19), and AD is also strongly associated with mutations in the barrier protein, filaggrin (20). It has therefore been hypothesised that an abnormal epidermal

environment caused by a leaky skin barrier predisposes the skin to infection by exposing environmental niches that would normally be inaccessible to *S. aureus.*

Unravelling the role of the microbiome in dermal diseases is confounded by the physiological changes in host tissues that characterise the pathology. Acne vulgaris, for example, has been associated with overgrowth of *Cutibacterium* (formerly *Proprionibacterium) acnes*, but this association is not necessarily causal. In addition, Acne vulgaris has been potentially linked to changes in the dermal environment proposed to be driven by factors including a Western style diet, which may influence signaling in the hair follicle resulting in overproduction of sebum (21). The photodermatosis, polymorphic light eruption (PLE) that is characterised by a rash on exposure to UV light has been associated with the abnormal expression of antimicrobial peptides in the skin, (22) distinct from that seen in psoriasis or AD, suggesting a microbiota involvement. PLE is however also associated with other changes in the immune system of the skin (23) (24). The common inflammatory skin condition psoriasis has been associated with changes in the skin microbiota (25) (26) but this association is not necessarily causal because the massive systemic inflammatory response that is a feature of psoriasis may also profoundly influence the composition of the skin microbiota (as reviewed by (27)).

Whilst the relationship between the oral microbiome and oral disease is arguably better understood, knowledge gaps remain. Common conditions such as dental caries, gingivitis and periodontitis are closely associated with potentially harmful changes in the composition and activities of the oral microbiota (sometimes referred to as dysbiosis) (28) (29) that have environmental triggers. The development of caries for example, is related to high intake of sugary foods and the consequent production of lactic acid by caries-associated bacteria within the oral microbiome. This in turn favours the growth of acid-tolerant, acidogenic organisms such as *Streptococcus mutans* which, along with other oral bacteria, forms biofilms on the tooth surface (30). Acid produced by these organisms can alter the balance of enamel demineralisation/remineralisation of the tooth, leading to

loss of mineral, and caries formation. In periodontitis, the persistent presence of subgingival biofilms associated with poor oral hygiene can lead to inflammation and bone loss (31). The pathology of periodontitis is largely caused by the host response and the primary risk factor is host susceptibility (as reviewed by Wade (32)). However, certain species of bacteria favour inflamed sites including *Porphyromonas gingivalis*, which can subvert the host response leading to a "dysbiotic" microbiota, which further exacerbates lesions (33). Whilst the role of the host response in periodontitis is well established, the roles of host response and microbiome for gingivitis merits further research. Additionally, some reports suggest that oral bacteria can translocate from the mouth into the systemic circulation and whilst causality has not been confirmed, periodontitis for example, has been associated with other conditions such as coronary artery disease (34), rheumatoid arthritis (35) and respiratory disease (36) (37).

Targeting specific microbes with personal care products

As well as investigating the role of the microorganisms present in health and disease, microbiome research is increasingly being applied to investigate the fundamental biology of various skin conditions (38), oral hygiene (39), dandruff (40), dental caries (41), acne (42) and periodontitis (28, 29) (Table 1). Recent advances in this field include improved knowledge of the bacterial and fungal composition of the scalp in individuals with and without dandruff (43), and the identification of bacteria involved in axillary (44) and oral (45) malodour. In addition, the importance of bacterial strain variability in acne is also now appreciated; although the overall relative abundance of *C. acnes* is comparable between acne and healthy individuals, significant differences at the strain level have been observed (42). Manipulation of the compositional structure or function of skin and oral microbiomes can potentially counteract certain undesirable health conditions where use of probiotics, prebiotics and targeted antimicrobials may provide opportunities to restore the healthy microbial composition of the skin (46) and oral cavity (47) (48). Manipulating innate immunity of the skin and oral cavity is a another route through which this could be achieved (39) (49)

Aims and Objectives

Whilst differentiating between association and causality remains a key issue in microbiome research, the fact that in some cases interactions between the microbiome and the host play a role in health and disease has been established (as previously reviewed (50)). It is therefore important that the effect of personal care regimes on the microbiome receives adequate consideration. Understanding of the factors that cause fluctuations in the microbiome is likely to contribute to the development of novel approaches to understand potential links to undesirable health conditions, and to the identification of microbiome-based biomarkers. It is in this context that the U.S. National Academy of Sciences have discussed the need to incorporate interactions between the microbiome and chemicals in assessing human health risks associated with environmental chemical exposure (51). As understanding of the functional significance of the human microbiome progresses, and the exploration of host-microbial interactions advances, understanding the effects of intentional manipulation of the human microbiome in the context of human safety should be addressed.

In October 2016, a workshop was organised at Colworth Science Park in the UK including 31 specialists in the areas of microbial risk assessment, skin and the oral microbiome, microbial ecology, bioinformatics, bacterial modelling and immunology. This manuscript emerged from exploration of the areas discussed during the workshop. It considers factors that the panel agreed require consideration when evaluating the safety of personal care products that aim to benefit the consumer by affecting the composition or activities of the skin and oral microbiomes.

PROTECTION OF THE ORAL AND SKIN MICROBIOME FUNCTIONS TO PROMOTE HEALTH

The human microbiome in health and wellbeing

Microbiotas associated with the oral mucosa and the skin help programme the human immune system to recognise pathogens (52, 53), reduce the risk of invasion by undesired organisms (54), produce vitamins and other metabolites such as short-chain fatty acids (55). In skin, Phenol Soluble

Modulins (PSMs) and bacteriocins (56) contribute to the ecological and structural maintenance of the niche (54). Commensal skin organisms such as *S. epidermidis* and *C. acnes* use distinct mechanisms to inhibit pathogens and maintain a healthy skin barrier. *S. epidermidis* produces antimicrobial peptides which can reportedly control the growth of *S. aureus* (16) as well as serine proteases to inhibit biofilm formation (16), fermentation products such as succinic acid that may inhibit the overgrowth of the opportunistic pathogen *C. acnes* (46), and a unique form of lipoteichoic acid that can inhibit skin inflammation during skin injury (57). *C. acnes* has also a protective role as a commensal by converting sebum to free fatty acids, which in consequence inhibit colonisation of opportunistic pathogens and contribute to the maintenance of an acidic skin pH (46).

In the oral cavity, some streptococci generate hydrogen peroxide that can inhibit the caries-associated bacterium *S. mutans* (58). The oral microbiome also has non-antimicrobial functions of importance to health and disease where nitrate-reducing oral bacteria can convert dietary nitrate into nitrites, which can influence cardiovascular health and blood pressure (59). Nutritional functions of the oral microbiota are delivered by complex communities via cross feeding and syntrophy. For example, streptococci have both glycosidic and endopeptidase activity, whilst species of *Prevotella* and *Porphyromonas* species have endopeptidase activity and *Fusobacterium* and *Peptostreptococcus* have aminopeptidase activity (60). Bearing in mind the roles of the skin and oral microbiome that are currently understood and the fact that other activities remain unknown, the maintenance and protection of the healthy functionality of the microbiome is an important consideration when assessing the effect of personal care products.

Microbiome Composition *versus* function

Initiatives such as the Human Microbiome Project (HMP) (13) (53) (55) and other studies (14, 52, 61) have enhanced our understanding of baseline skin and oral microbial composition but the search for attributes that define a healthy human microbiome continues. As part of the HMP, where 200

healthy individuals were examined, the “core” microbiome of different body sites, including saliva, plaque, tongue and other oral tissues, ranged from zero to eight operational taxonomic units (OTUs) when analysed for percentage prevalence of 100% compared to a higher range of 19-75 OTUs when the percentage was lowered to 50% (62). Interpretation of the core microbiome to measure the similarity of samples depends on the taxonomic resolution employed since samples may decrease in apparent similarity when analysed to genus or OTUs compared to phylum level (61) (63) (64). Whilst a specific group of microorganisms may be shared between individuals, inter-individual variation may still be considerable at the species-level, and for the presence of rare microorganisms (8, 14, 61). Care is therefore required when classifying microbiome composition as healthy or otherwise, especially in the absence of species-level classification. This is of particular importance in the oral cavity where different species within the same genera can have contrasting associations between health and disease.

The functions provided by compositionally different microbiomes can be relatively similar between individuals (55). Exploring which of these general functions are associated with health represents an alternative to the concept of “healthy composition” (65). A proposed functionality-based definition of a “healthy microbiome” involves three functions: those associated with health-related housekeeping functions, human functions, and specialised functions (53). Housekeeping functions involve energy production and the generation of metabolites and other requirements to maintain the microbial community itself; human-associated functions comprise interactions with the host such as developing and influencing the activity of the immune system and specialised functions include regulation of the pH in a specific body site. A functional core has been described for metabolic pathways detected in more than 75% of individuals (55). Pathway cores were identified for either multiple or single body sites, reflecting the fact that some core functions are broadly distributed and general to the human host whilst others are an adaptation to a specific body site. It should be noted that core functions are not necessarily beneficial to the host. Among site-enriched pathways, nitrate

reduction has been identified as important in the oral cavity (55). These core pathways are generally associated with microbial consortia. Such functional observations may provide further insights when studied across populations and during longer temporal studies with a controlled microbial change. If functional characterisation of the human microbiome can be achieved, measuring or predicting the loss of a beneficial function or the introduction of an undesired function could be used as a functional index during consumer safety assurance.

FACTORS THAT CONTRIBUTE TO PERTURBATION OF THE SKIN AND ORAL MICROBIOME

Microbiome stability as an indicator of health

The stability of the microbiome over time in healthy individuals has been assessed (66). Temporal stability has been explained as a state of equilibrium for a community regardless of the fact that some microbes may at the same time be changing as response to disturbances (67). The ability of the microbiome to remain balanced when exposed to a perturbation and to recover to a healthy functional profile afterwards has also been proposed as a key feature of a healthy microbiome (53). Despite their importance for understanding microbial community dynamics and responses to perturbations, long-term longitudinal studies are still rare. However, based on the available evidence, the composition of the human microbiome is relatively stable over time, with the main variation within an individual being between body sites (13) and considerable temporal stability has also been reported for the microbiome in healthy skin. Oh and colleagues (68) generated metagenomic sequence data from longitudinal samples collected over 2 years and reported that bacterial, fungal, and viral communities were largely stable over that time despite exposure to the external environment. This stability was observed to be site-specific, with body sites harbouring high microbiome diversity being more variable than low diversity (sebaceous) body sites. Observations of temporal stability in the skin microbiome have been interpreted as evidence for colonization resistance and used as the basis for clinical studies exploring skin microbiome in

disease states, where compositional changes in the microbiome have been reported. Costello *et al.* (10) assessed the resilience of the skin microbiota by disinfecting plots on the forehead and left volar *(i.e.* underside of) forearms of volunteers and then inoculating them with "foreign" microbiotas *(i.e.* taken from the tongue and skin of other individuals). The microbiotas of forearm plots (n= 16) that had been inoculated with tongue scrapings were more similar to tongue communities than to those normally associated with the forearm in relative abundance between 2 and 8 h after inoculation. However, communities more similar to those normally associated with the forehead, developed on forehead plots that had been similarly inoculated with tongue material. It can be inferred therefore, that for some reason (potentially the presence of sebaceous lipids), the forehead environment exerted a stronger selection pressure than the forearm. Furthermore, following interpersonal and inter-gender reciprocal swaps of forehead and forearm microbiotas, developing communities resembled the recipient rather than the donor, demonstrating the importance of the environment and possibly, the action of endogenous mechanisms for individualisation and microbiota perpetuation. The authors hypothesised that the stronger selection at forehead sites was due to sebaceous secretions which, in contrast to dry sites like the volar forearm, may have i) been more strongly selective and/or ii) could have supported the more rapid recolonisation from appendageal structures, which is in agreement with the hypothesis outlined above.

The oral microbiota may also remain stable over time in healthy individuals (6) although it is also sufficiently malleable to be beneficially manipulated through hygienic intervention (39). It is however important to consider what stability means when referring to a host-associated microbiota. Belstrøm and colleagues collected saliva from five volunteers without oral disease every 4 h for 24 h, repeated this seven days later (69) and profiled the salivary microbiome. Whilst caution is necessary given the small sample size, the author's tentative conclusion was that "little or no variation" within salivary microbiomes was observed over time. The oral cavity is a complex

285 environment with various distinct areas, and saliva, often purported to contain microorganisms
286 originating from multiple sites on the mouth may vary less in terms of microbiome composition
287 than for example, a tooth surface where in individuals following the recommended oral health
288 regime of twice daily brushing microbial abundance will be very low immediately after cleaning,
289 but can exceed 10^7 bacteria per cm^2 following regrowth.

290 Maintaining microbiome stability in healthy individuals will ensure that the beneficial microbial
291 functions are maintained (70) so the measurement of microbiome stability and its recovery
292 following disturbance are important in understanding potential risks. Whilst the human microbiome
293 is relatively stable, its composition can be altered both by pathologies such as gingivitis and
294 dandruff, or by treatment.

295 **Consumer products can alter microbiome composition or function**

296 The hypothesis that the skin microbiota, once established, is perpetuated by continuous endogenous
297 inoculation is supported by an investigation by Grice *et al.* (12) in which skin microbiota was
298 sampled using swabs and biopsies and profiled by high-throughput sequencing. An attractive
299 explanation is that secretions from sweat glands and the outward migration of differentiating skin
300 cells could transport bacteria cells from within appendageal structures continuously onto the skin
301 surface (as proposed by Kong et al. (71)). Daily hygiene regimens may however affect the
302 microbiome and some routines tooth brushing and hand washing do this intentionally to
303 respectively control reduce the risk of oral disease and to reduce the transmission of pathogens, (54,
304 72). Exposure to antimicrobials through the use of household and personal care products has shown
305 minimal long-term effects on the microbiome. In this respect, two human studies monitored how the
306 use of toothpaste, liquid and bar soap, and dishwashing liquid, with and without triclosan perturbed
307 the microbiome. The first study; a crossover control study involving healthy individuals, showed no
308 significant impact on human oral or gut microbiome composition during 4 months exposure to the
309 antibacterial compound triclosan (73). A longitudinal survey of the gut microbiota in infants and

mothers during the first year following birth also did not show major compositional changes or loss of microbial diversity (74). It is highly likely that environmental modulation of the skin microbiota has been occurring since the ancient origins of the microbiome for the skin through UV irradiation, friction and washing, and for the oral cavity through diet, friction and cleaning. In personal care, antiperspirants are used by approximately 50% of the global population and have been shown to reduce bacterial load in the axilla. Individuals that do not use antiperspirants have been observed to harbour greater axillary microbiome diversity than individuals that use antiperspirants do (75). For antiperspirant and deodorant users who ceased use of product, an increase in Staphylococcaceae was observed, in comparison to *Corynebacterium* species dominating in non-users. Perhaps surprisingly, microbiome diversity was reported to be greater in antiperspirant users compared to deodorant or non-users. In a separate study of nine cohorts, axillary diversity was similarly found to be greater in antiperspirant (and deodorant) users compared to non-users (76). A recent study on effect of cosmetic products on the microbiome of facial skin of high and low hydration groups indicated that baseline bacterial diversity was greater in the low than that of high hydration group, and that the use of cosmetic products decreased the differences between the two groups (38).

Microbiome individualisation

Evidence suggests that both environment and host genetics play important roles in determining the composition of individual microbiomes. Salivary microbiome studies in twins indicate that overall microbial abundance and some aspects of the microbial population structure are influenced by heritability (77). With respect to the skin microbiome, Blekhman and colleagues (78) analysed shotgun metagenomic data from the HMP, collecting data on host genetic variation for 93 individuals. They reported significant associations between host genetics and microbiome composition for ten of the fifteen sites they assessed, including the oral cavity and the skin. Thus, as well as extrinsic environmental factors, host genetics appears to play a role in the composition of the oral and skin microbiotas, probably through immunological and other mechanisms. These

examples partly explain the variability between individuals observed in microbiome research (8) and highlight the need to separate a significant change from individual variation when assessing specific perturbations.

Extrinsic factors also influence the stability of the microbiome since activities such as smoking tobacco have been shown to influence the composition of oral biofilms (79), suggesting that smoking promotes the acquisition and colonisation of pathogenic bacteria. The development of gingivitis and its progression from gingivitis to periodontitis and the promotion of dental plaque biofilm colonisation partly depends on the host immune response (80). Gomez and colleagues (81) illustrated the impact of host genetics through a human volunteer study involving a large cohort of monozygotic and dizygotic twin children with and without active caries, with the aim of elucidating the contributions of host genotype and shared environment on the oral microbiomes (supragingival plaque) of children. They observed that similarity in oral microbiomes was higher between monozygotic twins regardless of caries state, with certain taxa being identified as highly heritable but that most of the variation was determined by the specific growth microenvironment. The caries state however, was not associated with the more highly heritable bacteria suggesting that lifestyle, diet and oral hygiene practices might outweigh parental heritability in establishment of a caries associated microbiome. The more heritable species were detected at lower abundance with increasing age and sugar consumption.

MEASURING CHANGES IN MICROBIOME COMPOSITION AND ACTIVITIES

Risks of pathogen colonisation

One of the beneficial activities of the microbiotas of the skin and oral cavity is the protection of the host tissue from pathogens (as summarised in Figure 1). Perturbation of commensal communities may be therefore a contributing factor to the pathogenesis of certain inflammatory conditions. In some circumstances, overgrowth of commensal microorganisms with pathogenic potential (pathobionts) or colonisation by external pathogenic organisms (transients) can cause disease. The

ability of transient organisms to colonise is likely to depend on the interactions with the commensals residing at each specific body site. In this respect, microbial communities with more competitive interactions than cooperative interactions are assumed to be more resilient in the sense that cooperation causes coupling between species involving several species to change at the same time and destabilise the system (82). In the mouth, loss of colonisation resistance through antibiotic use can lead to infections by opportunistic pathogens such as *Candida* species and *S. aureus* (as reviewed (83) (60)). In this regard, microbial changes that do not increase the opportunity for pathogens to colonise are unlikely to adversely affect the wellbeing of the host.

The human body as a microbial niche

The skin and oral cavity present distinct environments, and ecological conditions *in situ*, have a large influence on the compositional differences in microbiota between body sites. Oily, moist and dry skin sites regulate nutrients and harbour specific microbial taxa (46, 52, 84). The mouth can be broadly divided into different habitats: the gingiva and hard palate; the tongue and throat; and dental plaque; each one colonised by a microbiome characteristic of the specific site (60). The microbiota present in the oral cavity form biofilms by attaching to the different surfaces, which confer spatial structure and provide the conditions required for different organisms to survive within the community (85). The availability of oxygen is one of the drivers of microbiota composition and in this context, a succession during the formation of dental plaque has been proposed whereby teeth are initially colonised by facultative genera such as *Streptococcus*, with a shift to a microbial community better adapted to anaerobic conditions, as the biofilm matures. Bacterial succession on the tooth surface can also be strongly influenced by nutrient availability, mechanical stress and saliva flow (6, 61, 86) and by binding of bacteria to proteins in the salivary pellicle coating the tooth surface (87).

Interactions with the external environment can also drive selection. For example, an increase in sugar intake or a reduction in saliva flow may induce a reduction in pH that allows the expansion of

aciduric organisms (86). Loss of moisture, changes in temperature and exposure to ultraviolet radiation can also result in microbiota alteration in the skin (88). Similarly, changes in the spatial structure may also influence the microbial community within a given body site (9, 88).

Microbial diversity in health

Several indices have been employed to differentiate microbiomes associated with health and disease. Among these, microbial (ecological) diversity is frequently measured. Ecological diversity can be measured as richness (the number of taxa present) and evenness (the abundance of microbial constituents). Although not universally applicable, higher diversity has been associated with health in specific contexts when considering that more diverse microbes may supply the host with increased functional traits. However, microbial diversity on its own is not an accurate measure for determining disease aetiology or health. Whilst reduced microbial diversity has frequently been observed in conditions such as atopic dermatitis and psoriasis (89)-(90) this is not always the case, for example, in both psoriatic and unaffected elbows (81) richness has been reported to be the same whilst, an increase in bacterial diversity due to the rise of species of minor abundance has been observed in gingivitis and periodontitis (64, 91). The measurement of diversity also does not account for interactions among species and two microbiomes with the same level of diversity may be different. It may therefore be more pertinent to observe the entire community of microbes present and by extension how they are functioning, rather than relying on richness alone as a predictor of disease (92).

The importance of bacterial abundance

Compositional studies of the skin and oral microbiomes have suggested that the load or abundance of organisms can be more significant than their presence in the progression of disease. A 65% increase in the proportion of *S. aureus* in atopic dermatitis sufferers at flare sites and partial correlation between *S. aureus* abundance and disease severity have been reported (99). Similarly, *S.*

411 *epidermidis* was significantly more abundant during flares than post flares and in controls, although
412 the underlying reasoning for the increase in *S. epidermidis* was not determined (99). Several studies
413 have reported increased *C. acnes* abundance in acne compared to unaffected volunteers (93). Whilst
414 differences between the absolute numbers of bacteria between inflammatory acne, papules and
415 pustules have been reported there appears to be progressively higher bacterial loads vis-à-vis
416 severity of the disease (94). The use of quantification methods such as quantitative PCR has
417 revealed higher levels of *S. mutans* and *S. sobrinus* in children with caries compared to caries-free
418 children (95). In other oral diseases such as gingivitis, severity is better correlated with the plaque
419 load and maturity than with some specific bacteria (60). It should however be born in mind that
420 NGS is not well-suited to determining differences in bacterial absolute abundance (quantified
421 genetic or microbial load within a sample) such that two samples with identical relative abundance
422 (genetic representation of microbes within a sample ranked against all taxa in the sample) could
423 differ markedly in absolute abundance (96).

424 **Host-microbiota interactions**

425 Skin functions as a two-way barrier, which helps to preserve hydration levels and prevent entry of
426 noxious substances into the body. Skin function may be shaped by the commensal organisms and in
427 this respect, Naik *et al.* (97) demonstrated that germ-free mice had a weakened immune response to
428 the parasite *Leishmania major* compared to mice raised under specific pathogen-free conditions.
429 The impaired response in the germ-free mice could be rescued by colonisation with *S. epidermidis*
430 (97) implying a role for the microbiota in promoting host immunity. More recent evidence suggests
431 that the microbiota is fundamental to skin structure. Conventionally reared mice showed altered
432 gene expression compared with germ-free mice. Meisel et al. (98) reported that 2820 genes were
433 differentially regulated by microbial colonisation, which included genes associated not only with
434 the host immune response but also epidermal differentiation. Crucially, the expression of 9 genes
435 involved in the epidermal differentiation complex (EDC), a collection of genes involved in terminal

differentiation of keratinocytes (reviewed in (99)), was regulated by the microbiota. When the skin of conventionally raised mice was compared to germ-free mice, differences in the balance of proliferation and differentiation were observed. These data support the view that the microbiome may be associated with the development of the skin architecture since the EDC has been implicated in dermatological diseases such as psoriasis (reviewed in (100)). Various studies have shown that the microbiota is associated with the outcome of the healing response when wounding breaches the skin barrier. In broken skin the commensal microorganisms can behave as pathogens and colonisation of wound sites can result in release of microbial metabolites that can further damage host tissues (reviewed in (101)). It is therefore unsurprising that accelerated wound healing has been observed in the absence of microbiota (102, 103) but it is also the case that the commensal microbiota can produce antimicrobial peptides (AMPs) that can inhibit the invasion of wound sites by pathogens (104). There is also evidence that *S. epidermidis* can inhibit the uncontrolled inflammation sometimes associated with wounding. Part of the mechanism for this may involve the inhibition of cytokine release by keratinocytes (57).

With respect to beneficial effects, *S. epidermidis* has been reported to augment tight junction function in keratinocytes (105) where the interaction of keratinocyte monolayers with *S. epidermidis* increased the trans-epithelial electrical resistance (a measure of tight junction function) within a short time of exposure to this bacterium. Furthermore, toll-like receptor (TLR) ligands such as lipoteichoic acid or peptidoglycan may augment tight junction function in keratinocyte monolayers (106). These data suggest that skin commensals, like those of the gut, are probably involved in many aspects of epithelial barrier homeostasis.

MEASURING CHANGES IN MICROBIOME COMPOSITION

Various data analysis methods are used in microbiome research that can objectively assess microbial changes. This section describes the information that each technique provides and how it is applied to characterise health and disease.

Metagenomic profiling

Studies employing both ribosomal profiling and metagenomics have sought to identify microbes linked to either oral or cutaneous disease, whether at the community level or that of individual taxa. Several studies have reported changes in the proportion of bacteria on the skin in psoriasis (25, 26, 89). Gao et al (25, 26) for example reported that Firmicutes were significantly overrepresented in psoriasis lesions compared to uninvolved skin, whilst the *Actinobacteria* and *Propionibacterium* species were reportedly present at significantly lower relative abundance in psoriatic lesions. Apart from bacteria, the fungal genus *Malassezia* has also been associated with psoriasis (89, 107-110). Altered microbial community profiles have also been reported in atopic dermatitis, where an increased proportion of *Staphylococcus*, particularly *S. aureus* and *S. epidermidis*, were observed during disease flares in comparison to baseline or post-treatment, and correlated with increased disease severity (111-113).

In terms of the oral microbiota, changes in microbial composition have long been associated with dental caries and periodontitis. For caries, sequence analysis has confirmed that bacteria other than *S. mutans* are correlated with active caries *(Lactobacillus* and *Bifidobacterium)* and likewise several taxonomic groups of bacteria are associated with periodontitis (28, 114-117). It is also clear that the aetiology of disease also involves a complex interplay between the host and the resident microbial communities that is yet to be fully explored. Applied to the study of psoriasis, such approaches indicate that strain level features and associated functional variation may be pertinent to disease (118).

This exploration of host-microbe interactions have been hindered by the fact that virulence and pathogenic determinants could be partitioned at the sub-species or strain level. It is well established that intra-species genomic features lead to phenotypic variability (113, 119-121). Ribosomal genera-based profiling approaches lack strain level resolution. Several recent computational tools to

taxonomically (122-124) and functionally (125, 126) characterise individual members of the microbiome at strain level resolution in metagenomic datasets have become available.

Profiling of functional potential

Whilst understanding the community structure of a microbiome and the relationship between specific taxa and health or disease can be informative, knowledge of community function will probably be most useful in understanding the effect of perturbing the microbiome. Shotgun metagenomics provides the potential to access strain level taxonomic features and the potential functional characteristics of the community which has until recently been computationally challenging. This approach can be used for the investigation of functional traits, although it can only reveal the functional potential of communities. It can also be used to profile viruses, which are not amenable to ribosomal-based profiling. The oral microbiome have assessed disease states such as caries or periodontal disease compared to healthy controls. Shi *et al.* (127) and Wang *et al.* (128) reported that community function around bacterial chemotaxis and cell motility are increased in disease compared to periodontal health. It has also been shown that in periodontal disease there is an increase in metabolic pathway genes associated with fatty acid metabolism (129), as well as an increase in genes associated with the metabolic degradation of nutrients (127) and those required for growth in anaerobic conditions (129). Healthy communities have been shown to exhibit increased functions in the areas of fatty acid biosynthesis, aspartate and homoserine metabolism, membrane transport and signal transduction. Metagenomic studies of the skin are more difficult due to the low bacterial density and small sample surfaces available (130). Mathieu *et al.* (131) consider the skin microbiota as a complete organism, reporting a predominance of catabolic genes and the ability of the skin bacteria to use the sugars, lipids and iron that are found on human skin. They also found genes related to antibiotic resistance, as well as some linked to acid resistance, clearly a mechanism for tolerance of the natural acidity of the skin. Oh *et al.* (17) have described a “functional core” of around 30% of the community that can vary depending on the diversity and biogeography of the

differing skin microenvironments, which drives the functional capacity that is required by that community. For example, dry sites were found to favour functional traits surrounding the citrate cycle, and sebaceous sites showed increased function around glycolysis and ATP/GTP/NADH dehydrogenase I. Whilst these metagenomic approaches exceed a simple inventory of taxa and provide information on function and health/disease interrelationships, making judgements of community functional traits by reference genome comparison should be undertaken with care. There is a large genomic diversity that is just starting to be understood, for example the association of only some *C. acnes* strains with acne vulgaris (123) (130). Further complicating the search for a functional understanding of the microbiome is the identification of new genes from metagenomic analysis approaches that are associated with health or disease, but which cannot be assigned to any functional pathway.

Metatranscriptomic analyses

Shotgun transcriptomics can be used to determine the active functions of a microbiome (132), especially as the community composition of a microbiome alone is not necessarily reflective of its active community members (133). This is an emerging research area with less data available, and challenges remain, for example in sampling sufficient mRNA material to enable analysis. However, the transcriptomic profile of a community is dynamic and can easily change in the same biological sample at different times as the microbiome responds continually to changing environmental and host conditions. Metatranscriptomic studies applied to human microbiome are more limited in comparison to metataxonomic/metagenomics surveys.

In comparison to the oral microbiome, metatranscriptomics of the skin is more challenging due to the limitations of microbial biomass in the sample material. Kang and colleagues (132) analysed the metatranscriptomics of patients with acne vulgaris versus healthy controls. *C. acnes* was reportedly the most transcriptionally active organism and was predominant in both the healthy and diseased samples. Further analysis of the gene expression profile of *C. acnes* in the samples identified that

535 the organism's activity on acne-affected skin was distinct from its activity on healthy skin.
536 Specifically, vitamin B12 biosynthesis pathway was observed to be significantly downregulated in
537 acne. Additionally, a model of how vitamin B12 modulates the transcriptional and metabolic
538 activities of *C. acnes* in acne pathogenesis was suggested. The model underlined how shotgun
539 metatranscriptomic approaches can enhance the understanding of disease pathogenesis. One of the
540 limitations of meta-transcriptome data is the final metabolic products generated by a microbial
541 community are not captured (133). In this respect, techniques such as proteomics, metabolomics,
542 and lipidomics can help to have a deeper functional characterisation of the microbiome.

543 Metatranscriptomics has been used in conjunction with metagenomics to investigate saliva from
544 individuals with caries and periodontitis to compare with saliva from orally disease-free individuals.
545 Belstrom *et al.* (69) identified 15 differentially expressed KEGG Orthologs (KOs) between
546 periodontitis or caries samples when compared with orally healthy controls. These included eight
547 carbohydrate metabolism-associated KOs that were downregulated in periodontal disease and two
548 KOs that were upregulated in caries associated with glycan biosynthesis and carbohydrate
549 metabolism. In addition, the same study observed that lipid metabolism was increased in healthy
550 samples when compared with dental caries and concluded that longitudinal studies may reveal that
551 screening salivary metabolic gene expression can identify oral diseases preclinically. However, it is
552 also clear that development of such diagnostics is at a very early stage and that overcoming the very
553 significant differences in complexity between the salivary and plaque microbiomes would be a
554 substantial technical and clinical challenge.

555 **Metabolomic analyses**

556 Microbial metabolites can have a direct impact on oral or skin health (*e.g.* short chain fatty acids
557 and sulphides in periodontal diseases, organic acids in dental caries) or they can enter and modulate
558 host metabolic processes. As such, metabolite exchange between the microbiome and host

559 represents one mechanism through which these systems communicate. Variation in the bacterial
560 species present can modulate the genetic library of the microbiome, changing its overall functional
561 capacity, its metabolite production, and the downstream impact on host health. However, different
562 species are known to possess similar or even the same metabolic traits. This functional redundancy
563 means that studying composition alone may be insufficient to accurately determine the overall
564 biotransformation capabilities of the microbiome and therefore its potential to modify host health.
565 Metabolic profiling (metabolomics/metabonomics) has emerged as a powerful tool for studying the
566 microbiota because it can ascertain the metabolic profile via low molecular weight compounds in a
567 sample. These metabolic signatures contain thousands of molecular small molecular weight
568 compounds reflecting biochemical events. This includes host metabolic processes but also those
569 performed by the resident microbes and products arising from interactions between the two. Studies
570 using metabolomics to directly assess the functional status of the skin microbiota are limited.
571 However, several studies have characterised the skin metabolome in a wider context. These have
572 used a variety of sample types including skin swabs, hydrogel micropatches (134), punch biopsies
573 and sweat. In one study analysing epidermal skin tissue, several bacterial-derived metabolites (135)
574 and bacterial substrates were observed, including *p*-cresol, a bacterial metabolite of tyrosine. This
575 demonstrates that these tissue samples can be informative for studying the skin microbiome. Skin
576 surface liquid extracts (sweat) represent another sample type of potential utility. These are complex
577 mixtures of secretions derived from eccrine, apocrine and/or sebaceous glands (depending of body
578 location) as well as from the microbiota inhabiting the skin (136). Attempts are being made to
579 optimise and standardise the collection and analysis of sweat and this may prove to be a useful
580 resource for studying the skin microbiota.

581 Metabolic profiling of gingival crevicular fluid (GCF) has been used to study the importance of
582 host-bacterial interactions in periodontal disease. Here, the depletion of anti-oxidants, degradation
583 of host cellular components and accumulation of bacterial products were seen in the disease state

584 (137) (138). Attempts have been made to integrate salivary bacterial and metabolic datasets to
585 identify metabolic products related to specific bacterial groups (139). Oral biofilms have also been
586 studied by capillary electrophoresis-mass spectrometry (CE-MS)-based metabolomics. This has
587 enabled the central carbon metabolic pathways to be investigated in the oral biofilm. One approach
588 is to measure these pathways in supragingival plaque before and after a glucose rinse. Glucose can
589 be degraded by bacteria to several metabolic products, including acetate, formate, lactate, and
590 succinate. Assessing the metabolic content of this plaque after the rinse provides information on the
591 functional capacity of the biofilm.

592 **Mathematical modelling**

593 Oral and skin microbial community dynamics are shaped by three broad factors: the host, the
594 environment and the community. The human host provides the microenvironment for the
595 community and may alter this environment through hygiene and other behaviours. The genetic
596 makeup of the host also influences the community's microenvironment. The surrounding
597 environment offers a large species pool from which immigration into the local community may take
598 place. Finally, community composition (richness, evenness and interactions) as well as history (e.g.
599 previous exposure to perturbations) may impact its dynamics.

600 A community model expresses in mathematical terms how selected factors influence community
601 dynamics. Community models thus allow prediction of the response of the community to short-term
602 (pulse) perturbations and altered conditions (press perturbations). Models can be coarse-grained or
603 detailed, describing populations or individuals. A general distinction can be made between
604 phenomenological models that predict community behaviour on the basis of immigration and
605 mortality rates, interaction strengths, growth rates and other parameters, and metabolic models that
606 take underlying molecular mechanisms of interactions into account. The generalized Lotka-Volterra

607 equation and its variants (140-142), but also individual-based models such as the neutral model
608 (143) and its extensions are examples of the former.

609 In the oral cavity, these models have to deal with the complication that most community members
610 can exist in both a free-floating planktonic state, as well as part of a biofilm, which may have
611 different growth rates, different access to nutrients and engage in different interactions. Previously,
612 Schroeder and colleagues (144) proposed a discrete and continuous version of a model that
613 describes the dynamics of both planktonic and sessile communities in drinking water pipes and
614 which may be adapted to model community dynamics in the oral cavity. The programming
615 language “gro”, which was designed for individual-based modelling of spatially structured
616 microbial communities, may also be of interest in this respect (145). This facilitates the modelling
617 of cell behaviours planktonically or in microcolonies or biofilms. A range of factors including
618 growth rates, cell-signalling, diffusing and chemotaxis can be factored in.

619 Metabolic models require the accurate reconstruction of each community member's metabolism
620 (146), which is a major hurdle because of lack of reliable and complete genome annotations and the
621 large percentage of unknown gene functions. Metabolic reconstructions may be quickly generated
622 automatically with tools such as ModelSEED or RAVEN (147) (148). This type of modelling
623 present some disadvantages such as the requirement for a tedious manual curation to ensure an
624 accurate reconstruction (149) and the assumption that community members are in a metabolic
625 steady state. This assumption is relaxed by some dynamic metabolic models which require kinetic
626 parameters such as compound uptake rates (146). The dynamic individual-based metabolic
627 modelling tools COMETS (150) and BacArena (151) additionally take spatial structure into
628 account, which is important to model biofilms. Metabolic models can also integrate meta-omics
629 data as additional constraints on metabolic fluxes (152). For example, gene expression data has
630 been used to validate metabolic models (153). Despite their promise, to the best of our knowledge,
631 metabolic models have only been applied to communities consisting of a small number of species.

Metabolic models of species grown alone and in pairs can be exploited to predict ecological interactions (154). For instance, gut microbial interactions were predicted based on the semi-curated reconstruction of 773 gut species (155). The extension of dynamic and spatial metabolic models to more complex microbial communities is a promising field for future research.

Community-level metabolic networks are a simpler form of metabolic models, where metabolites and reactions are represented as nodes and edges, respectively, but where stoichiometric coefficients are not taken into account (156). They offer a framework for the straightforward integration of meta-omics data as node or edge weights (157). While metabolic networks can handle larger communities, they do not allow quantitative modelling (158).

Quantitative community models have parameters, which need to be determined through measurements in well-controlled conditions. For instance, growth assays in mono- and co-culture can provide growth rates and interaction strengths. Once a model is parameterized, it needs to be validated experimentally. Such a validation consists of comparing the outcomes of experimental perturbations with the outcomes predicted by the model. The model may undergo several rounds of adjustment and validation until it reaches sufficient accuracy, or it may fail to be predictive because important but unknown factors are not taken into account or the community dynamics are chaotic or predominantly stochastic. A model that predicts community dynamics to an acceptable level of accuracy can be applied to simulate the effects of yet untested perturbations on the community.

CONCLUSIONS

Perturbations of the microbiome can have positive and negative consequences for human health. However, more knowledge is required to understand the extent of change that corresponds to the maintenance of health and the establishment of disease states. Microbiome research is still in its early stages and further studies to elucidate the nature of the functional and structural interactions

among microorganisms and with the host are required. Analysis of the gut microbiome is advancing faster than that of the skin and oral microbiomes, where increasing research investment would help to understand better the dynamics of those two specific body niches. Although mankind has been manipulating its microbiome, often beneficially, through diet, hand washing and oral hygiene practices both modern and historic, for hundreds if not thousands of years, the risks of manipulating the microbiome through new technology innovation should be properly assessed and the development of appropriate methods is required. Numerous factors should be considered when assessing the safety of novel approaches to microbiome perturbation, and approaches need to be developed to ensure that a compositional change delivers benefits whilst not compromising the stability, diversity and immunological state required for healthy functionality of the microbiome. These are summarised in Table 1 and Figure 1. To increase our understanding of the safety of microbiome changes, multi-disciplinary research needs to move to a mechanistic understanding to allow measurable elements specific to the oral and skin microbiome to be identified.

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AUTHOR BIOS

Andrew J McBain is a Professor of Microbiology at the University of Manchester, UK. He was awarded his PhD from the University of Cambridge where he studied the human intestinal microbiota and pro/prebiotics with the Medical Research Council at Addenbrookes Hospital. His interest in the human micobiome has diversified since moving to Manchester in 1999, to include body sites such as the skin and the oral cavity. He also maintains research programmes in other

areas of applied microbiology and particularly enjoys multi-displinary research. He has supervised 29 PhD graduates and published over 100 papers.

Catherine A O'Neill received both her bachelor's degree and PhD from the University of Wales, Bangor where she studied bacterial biochemistry. Subsequently, she was a research fellow at the University of Leeds for 5 years before securing her first tenured appointment as a lecturer at the University of Manchester. Subsequently she was promoted to senior lecturer and then finally to Professor of Translational Dermatology, the post she currently holds at the University of Manchester. Professor O'Neill's interests are in using bacteria and their products for the treatment of skin in health and disease. The laboratory has a very translational focus and has a record in translating basic findings into human studies via the technology transfer into commercial vehicles. Professor O'Neill has been involved in this area since 2011.

Alejandro Amezquita PhD, graduated from the University of Nebraska-Lincoln (US), with more than 20 years of experience in various positions in academic (North Carolina State University, US) and industrial research (Unilever), currently working as Science & Technology Director within Unilever's R&D group, interested in microbiome innovation in consumer goods and risk-based approaches to assure product safety because of the importance of balancing the efficacy-safety continuum, using safety-by-design approaches as the foundation for safe innovation, He has been working in the microbiome innovation field for 4 years and in the consumer safety and microbiological risk assessment fields for 15 years.

Laura J Price received her Applied Biology BSc (Hons) from Staffordshire University in 2001. She started her career in Microbiology Quality Assurance for CAMR in 2002. For the following two years, she was a Leukaemia Research Associate for the MRC. Laura started working at SEAC Unilever in 2004, where she is currently a Microbiology Risk Assessor. Her role is to independently assess the consumer safety of new technologies and formulations designed by Unilever R&D. With the increasing interest in the microbiome as a target for consumer products designed to improve health and wellbeing, she is part of the Human Microbiome project, which is developing knowledge on how best to safety assess new technologies. Over the last 4-5 years the project has delivered a risk assessment framework, methods and data. The interactions of the microbiome and immune system, and dysbiosis manifesting as human disease, are what particularly interest her.

Karoline Faust is a biologist turned bioinformatician who graduated at the Humboldt University in Berlin and earned her PhD at the Université Libre de Bruxelles under the supervision of Prof. van Helden. She worked as a postdoctoral researcher at KU Leuven and VIB in the group of Prof. Raes. She is currently an Assistant Professor, heading the group of Microbial Systems Biology at KU Leuven since 2016. Her main research interests include the construction and analysis of microbial networks, the analysis of microbial sequencing data and the investigation of microbial community dynamics in silico and in vitro. She therefore works at the intersection of microbial ecology, systems biology and bioinformatics.

Adrian Tett is a Senior Research Associate in the Computational Metagenomics group (CIBIO, University of Trento). He received his Ph.D. from the NERC Centre for Ecology and Hydrology-Oxford in partnership with Cardiff University. As a Microbiologist and Bioinformatician he performed postdoctoral research at the BBSRC funded institutes, the John Innes Centre and the

Institute of Food Research. His current work focusses on the microbial communities and subspecies strain-level determinants associated with human health and disease. He is also developing novel approaches to explore the population structure, evolutionary history and subspecies diversification in abundant yet poorly characterised members of the human microbiome.

Nicola Segata Ph.D., is Associate Professor at the CIBIO Department of the University of Trento (Italy). He earned his Ph.D. in Computer Science at University of Trento in 2009 and he then moved to Harvard School of Public Health for his post-doctoral training where he started studying the human microbiome with computational metagenomics approaches. He came back to University of Trento (Department CIBIO) where he started his laboratory in 2013. His laboratory employs experimental meta'omic tools and novel computational approaches to study the diversity of the human microbiome across conditions and populations and its role in human diseases. His work is supported by the European Research Council and by several otther European agencies. The projects in his laboratory bring together computer scientists, microbiologists, statisticians, and clinicians and focus on profiling microbiomes with strain-level resolution and on meta-analysiing very large sets of metagenomes with novel computational tools.

Jonathan R Swann obtained a PhD in Biochemistry from the Department of Biomolecular Medicine at Imperial College London in 2008. Following his PhD, Dr Swann continued as a research associate at Imperial College in the area of molecular epidemiology. In 2010 he joined the School of Chemistry, Food and Pharmacy at the University of Reading as a Lecturer in Metabonomics. In this role, he developed metabolic phenotyping strategies to study the impact of nutrition, the gut microbiota, and parasitic infections on mammalian health and disease. In 2015, Jonathan joined the Division of Computational and Systems Medicine at Imperial College as a Senior Lecturer in Human Development and Microbiomics. He was appointed Associate Professor in 2017. He leads a metabonomic-based research programme to understand the influence of gene-environment interactions on the mammalian metabolic system and their implications for development, health and disease. His research has a specific focus on the microbiome.

Adrian M Smith was awarded a BSc in Biomedical Sciences from Sheffield Hallam University in 2001 and an MSc in Bioinformatics from the same institute in 2002. He worked briefly for GSK before taking up his current position as Bioinformatician for Unilever R&D in 2005. He has had an interest in Microbiomics for 9 years due to the initial disruptive nature of the science, and the speed at which it continues to develop and reveal previously hidden microbial secrets. Most recently he has had a particular focus on the development of bioinformatics analysis pipelines and visualisation tools for microbial ‘Omics data analysis.

Barry Murphy has received education at University College Dublin with Post-Doctoral studies at the University of Leicester encompassing microbiology, molecular biology and chemistry. A move to industry saw him establish and manage DNA sequencing laboratories across Europe before moving to Unilever to lead the microbiome capability group. Having held this position for 5 years he has an interest in understanding human associated microbial communities to investigate links between microbial metabolism and cosmetic conditions.

Mike Hoptroff is a senior project manager at Unilever with responsibility leading Microbiome Science and Technology in the UK. He graduated in 1995 from the University of Sheffield and then moved to research posts in the UK and USA prior to joining Unilever in 1998. Since joining Unilever he has spent 21 years in Microbiology R&D initially as a research scientist and subsequently as a project manager. During this time he spent approximately 6 years working on

skin cleansing and hand hygiene (2003-2008), 7 years on scalp microbiology (2009-2016), including 4 years leading Microbiology R&D in Unilever China and 3 years on Oral Care microbiology research (2016-). Michael has 13 peer reviewed publications and has led the market delivery of numerous product technologies.

Gordon James originates from Glasgow in Scotland, and was educated at University of Glasgow, graduating with a BSc and PhD in Biochemistry in 1987 and 1991, respectively. He then did a postdoctoral fellowship at University of Strathclyde in the area of environmental biotechnology, during which time he began practicing his favoured disciplines of microbiology and biochemistry. Gordon joined Unilever R&D in 1993, and in the time since, his main focus has been using his microbial biochemistry skills to probe the human skin microbiome, mainly to unravel the origins of axillary (underarm) odour. His current role is to provide scientific leadership to a UK-based team specialising in this topic on behalf of Unilever's Deodorants category and the global Science & Technology Platform, Human Microbiome.

Yugandhar Reddy is a Research Scientist with Beauty & Personal Care, Unilever R&D. I received my BSc and MSc in Microbiology and later Ph.D at the Indian Institute of Science, Bangalore. I was a postdoctoral fellow at the department of Microbiology & Molecular Genetics at University of Pittsburgh. Prior to joining Unilever, I worked as a Genomics Applications Scientist at Agilent Inc. My current interests are the Human Microbiome and its relevance for human health and wellbeing as well as building in vitro models to understand microbial community behaviour. In a previous role at Unilever I worked at the Safety and Environmental Assurance Center of Unilever Plc where I was exploring methods and approaches to risk assess Microbiome related technologies and led an S&T program on Microbial Ecology. I have been in this field for about 7 years to date.

Anindya Dasgupta has a PhD in Molecular Biology, Albert Einstein College of Medicine, New York, USA and is based at Unilever R&D, Bangalore. He is currentjy exploring scientific insights that play a crucial role in skin microbiome. The generation of these insights also help in screening of actives and development of products that have a positive impact on the skin microbiome. A key factor in this activity is to look at the safety aspect of microbiome modulation.

Tom Ross is a Professor in Food Microbiology at University of Tasmania. He was awarded his PhD from the University of Tasmania in 1994. Since thne he has been employed at University of Tasmania since 1994 as a researcher and teacher concerned with the quantitative microbial ecology of foods, and leading to my current position. He has supervised ~25 PhD graduates. He has published >150 international peer reviewed papers/book chapters with his students and colleagues. His research has also led to numerous software tools that translate his research into 'decision-support' tools for food safety and preservation that are used by governments and industry internationally. Those software tools are risk-based, and quantitative. He has been invited to contribute to many FAO/WHO scientific expert panels concerned with microbial food safety risk assessment. This background in quantitative risk assessment and microbial ecology led to his interest in the potential to modify the human skin microbiome and to assess the potentially associated risks.

Iain L Chapple is Head of the School of Dentistry; Research Director of the Institute of Clinical Science, Birmingham University, UK. He graduated 1986 from Newcastle University. Iain is former Scientific Editor of the British Dental Journal; Associate Editor of Journal of Periodontal Research and current Associate Editor of the Journal Clinical Periodontology. He has written 8-textbooks and 18 book chapters. Iain served the IADR Periodontal Research Group (PRG) as President (2006-7); Group Chair (2008-1015); Counsellor (2016). He served the European Federation of Periodontology (EFP) as: Treasurer (2007-2013); Workshop co-chair (2008-current); Chairman of Scientific Advisory Committee; Editor JCP Digest (2014-2016); Secretary General (2016-2019). He was British Society of Periodontology President 2014-2015 and awarded the Tomes medal - Royal College of Surgeons (2011); the IADR PRG Rizzo Award (2001); IADR Distinguished Scientist in 2018; Special citation award -American Academy of Periodontology 2018. Iain has >200 peer reviewed manuscripts in the international literature.

William G. Wade obtained his BSc in Biological Sciences at the University of East Anglia and a PhD in Microbiology at the University of Wales. He began his career as a Lecturer at the Welsh National School of Medicine in Cardiff and then moved to a Senior Lecturer appointment at the University of Bristol. He was appointed to the Richard Dickinson Chair of Oral Microbiology at UMDS (subsequently King's College London) in 1996. In 2013 he moved to Queen Mary University of London but returned to King's College London in 2018 to take up his current post of Professor of Oral Microbiology within the Centre for Host-Microbiome Interactions. He has played a major role in the characterisation of the oral microbiome, culture of previously uncultivated bacteria and the development of novel agents for the prevention and treatment of oral diseases. He has been active in microbiology research for 40 years.

Judith Fernandez-Piquer received her BSc in Chemical Engineering and BSc in Food Technology in Spain, her MSc in Food Safety in the Netherlands in 2007 and her PhD in Food Microbiology in Australia in 2012. Judith has a broad knowledge of risk assessment and the integration of predictive microbiology for exposure assessment in foods. After her PhD, she was involved in projects for Dairy Australia, Walnuts Australia and the Seafood CRC while at the University of Tasmania. Judith has a strong interest in protecting consumer’s health. She joined Unilever SEAC in 2014 as a risk assessor and led the Human Microbiome project, a programme that aims to enhance the safety assessment of microbial reprofiling to support innovative technologies in personal care. Judith started her current role as product safety manager with Upfield, a plant-based food company, in August 2018.

1296 TABLE 1. Habitat parameters, microbiome functions and intervention strategies for human skin and oral cavity

	Skin				Oral cavity		
Conditions with microbiome associations	Atopic dermatitis, psoriasis	Acne	Dandruff	Axillary malodor	Caries	Gingivitis	Periodontitis
Routine perturbations	Cleansing, moisturizing, use of cream, gels, lotions	Cleansing, use of cream, gels, lotions	Cleansing, use of shampoo	Cleansing, use of antiperspirants and deodorants	Toothbrushing, flossing, use of toothpaste, mouthwash		
Microbiome understanding and potential target mode of action for microbial interventions	*S. aureus* load correlates with atopic dermatitis flares (18) Early colonization with commensal staphylococci provides protection (REF 18, 19) Abnormal expression of antimicrobial peptides (22) Changes in the proportion of bacteria	Outgrowth of *C. acnes* and overproduction of sebum associated to acne (21, 93) Associated with specific strains of *C. acnes* (42, 119, 129) Decrease in the Vitamin B12 biosynthesis pathway (132)	Associated with an imbalance of both bacterial and fungal species, with an increase in *Staphylococcus* sp. and *M. restricta* (40). Severity of dandruff dependent on the interactions between the host and microorganisms (43) Decreased *Propionibacterium* and increased	Associated with *Corynebactrium* species (44) Malodour caused by short and medium chain volatile fatty acids (44)	Changes in oral microbiota composition (28, 29) Outgrowth of acid-tolerant *Streptococcus mutans* (30), *S. sobrinus* (96), *Lactobacillus* and *Bifidobacterium* (28, 114-117) Increased glycan synthesis and carbohydrate metabolism and reduced lipid metabolism (69)	Changes in oral microbiota composition (28, 29) Subversion of host response at inflamed site, colonization of inflamed tissue by *Porphyromonas gingivalis* (33) Plaque load and maturity (60)	Changes in oral microbiota composition (28, 29) Sub-gingival biofilm formation is associated with inflammation and bone loss (31) Translocation of oral microbiome to systemic circulation (34-36) Increased metabolic degradation of nutrients and fatty acid metabolism (126, 128) Increased gene

	compared to healthy skin (25, 26) Associated to the fungus *Malassezia* (106)	*Staphylococcus* abundance (43)	activity related to anaerobic growth conditions (128) Depletion of anti-oxidants, degradation of host cellular components and accumulation of bacterial products (136, 137)
Ecological factors specific to the human body site	Bacteriocins and phenol soluble modulins contribute to the maintenance of the niche (56) Skin has a mixture of secretions from different glands and microbiota (137) Host physiological conditions such as sebum and water content are relevant in scalp (43) Higher exposure to moisture, changes in temperature and UV (88) Host factors including skin barrier protein mutations e.g. Filaggrin in AD (20) and mTORC1 changes (increase sebum formation) due, in part, to diet (21) Host immune/inflammatory status (23 - 27)	Food intake, high intake of sugar correlated to production of lactic acid and acidification (REF 30,31) Biofilm formation by attaching to different surfaces (30, 31) Host susceptibility (32) Presence or absence of inflammation (33) Oxygen availability, mechanical stress and saliva flow (6, 61, 86) Antibiotic use (60) Exposure to tobacco smoke (79)	
Selected microbiota functions	*S. epidermis* produces AMPs to control the growth of *S. aureus* (16), serine proteases to inhibit biofilm (16) and fermentation products to inhibit *C. acnes* (46) *C. acnes* converts sebum to free fatty acids, inhibit colonisation and maintains acidic pH of the skin (46)	Some streptococci generate hydrogen peroxide to inhibit *S. mutans* (58) Nitrate-reducing bacteria can influence cardiovascular health and blood pressure (59) Some streptococci support enzymatic reactions for nutritional purposes (60)	

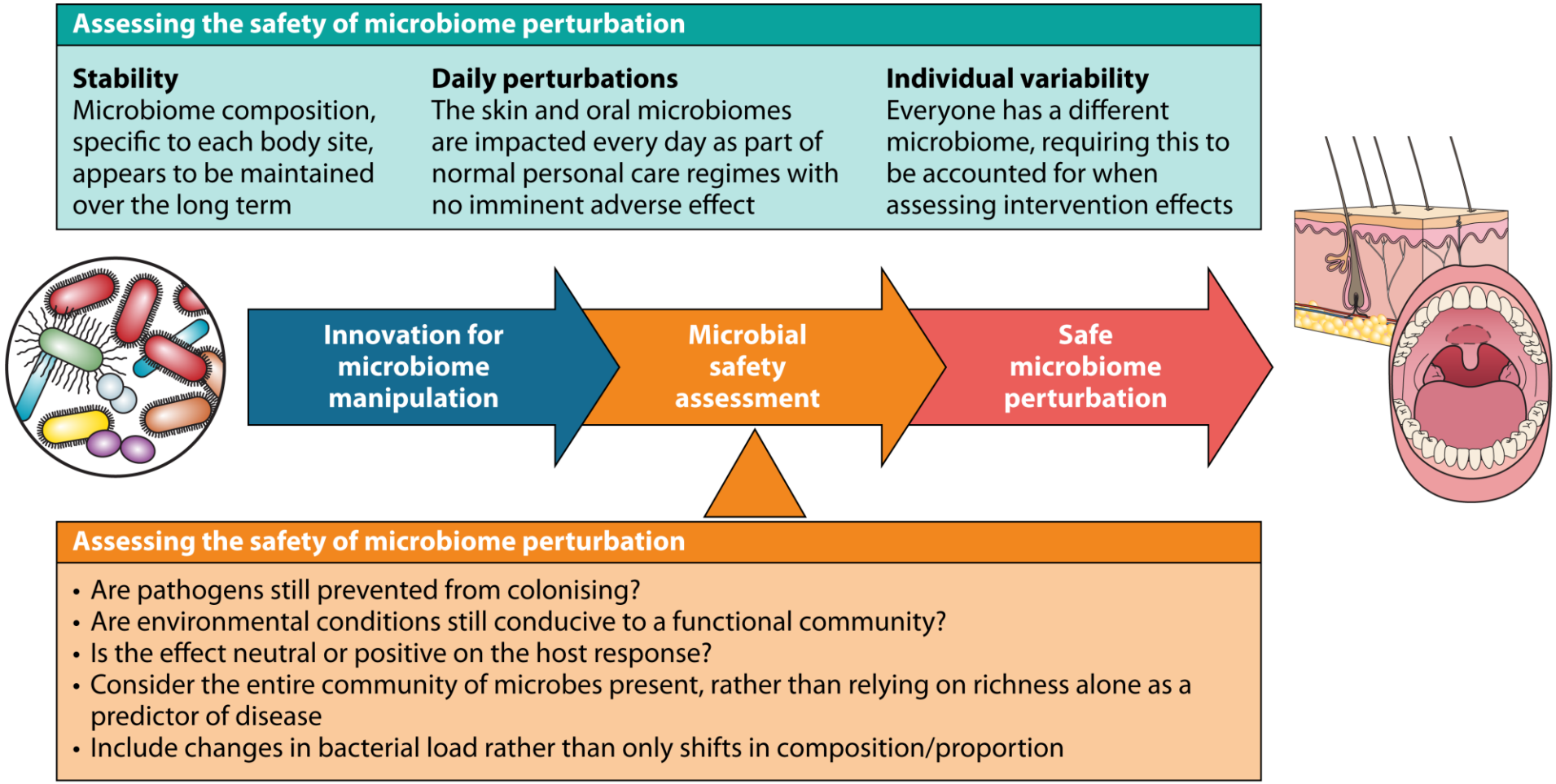

FIG 1. Assessing the safety of perturbations of the skin and oral microbiome

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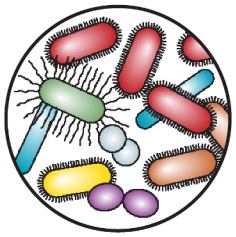
Assessing the safety of microbiome perturbation

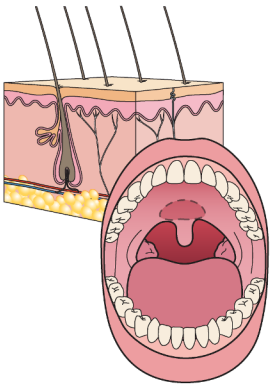
Stability
Microbiome composition, specific to each body site, appears to be maintained over the long term

Daily perturbations
The skin and oral microbiomes are impacted every day as part of normal personal care regimes with no imminent adverse effect

Individual variability
Everyone has a different microbiome, requiring this to be accounted for when assessing intervention effects



Innovation for microbiome manipulation → **Microbial safety assessment** → **Safe microbiome perturbation**



Assessing the safety of microbiome perturbation

- Are pathogens still prevented from colonising?
- Are environmental conditions still conducive to a functional community?
- Is the effect neutral or positive on the host response?
- Consider the entire community of microbes present, rather than relying on richness alone as a predictor of disease
- Include changes in bacterial load rather than only shifts in composition/proportion